EMBRYONIC DEVELOPMENT OF THE STABLE FLY, *STOMOXYS CALCITRANS*, LINNAEUS (DIPTERA: MUSCIDAE)- A LIGHT AND ELECTRON MICROSCOPY STUDY

by

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INTRODUCTION

The stable fly, Stomoxys calcitrans, is a widely distributed and reputedly vicious blood-sucking fly (Bishop, 1913). It is widespread in parts of the United States, especially the Gulf Coast states (Wright, J. 1972), and is the predominant fly species during certain seasons in Nigeria (Lyold and Dipeolu, 1974). As the so-called 'biting housefly' it is often a serious nuisance to man and, when preponderant, it vigorously enters the economic calculations of the livestock industry as a principal source of worry and a carrier (proven to be largely mechanical) of pathogens (Freeborn et al., 1925; Bishop, 1939; Baker and Quinn, 1978).

From a different perspective, however, the stable fly is gradually turning out to be a useful tool in scientific investigations of insect parasitism of livestock, which assumably require insect-animal models. The hematophagous habits (restless and intermittent type of feeding) characteristic of the fly (USDA, Agriculture Handbook #291, 1965), the ease with which it may now be reared in the laboratory (Bailey et al., 1975) and its susceptibility during various stages of its life cycle to a number of chemical and biological insect control agents (Wright, J. 1970) are some contributory factors to this increasing usefulness in laboratory and field investigations of aspects of human and veterinary entomology.

Notably there exists a sizeable amount of literature on this fly covering a range of subject matter. Earlier writings

included observations on the fly's metamorphosis and related subjects (Newstead, 1906; Austen, 1909; Bishop, 1913 and Hindle, 1914) as well as studies on bionomics (Mitzmain, 1913; Melvin, 1931; Simmons, 1944; and Kano, 1953). More recent works along the same lines include the Ugandan observations of Parr (1962); colonization and mass production data from McGregor and Dreiss (1955); Jones (1966) and Bailey et al., (1975); emergence pattern studies by Miller and Harris (1970), predictive models for development times of various immature stages of the fly from Berry et al., (1976) and the oviposition studies by Hoffman (1968) and Berry and Kunz (1978).

In addition, and as alluded to above, a large body of works now exists in which the stable fly had served as an insect model for various probes into aspects of insect parasitism of livestock. Such works include the attempt, early in the century, at transmitting poliomyelitis virus using the stable fly by Sawyer and Hermis (1913). Among others were the studies of percentage milk yield losses in lactating animals caused by fly annoyance (Freeborn et al., 1925) and the relatively recent employment of this fly as test insect model in the development of an assay to select candidate compounds with juvenile hormone activity (Wright, J. 1970).

The above mentioned volume of works not withstanding, however, there exists a tenous region in our store of information on this fly. This is with regard to its life cycle where detailed accounts, especially structural microscopy--of intrastage

developments are only thinly available. ("Stage" being used here as referring to the four developmental levels recognized in all holometaboly; egg--larva--pupa--adult). It must, however, be noted, that this paucity of microscopy information has been due, in part, to the difficulty generally encountered in preparatory procedures of insect specimen for microscopy; which latter is caused by the presence of insects' cuticular integument that hampers fixation, embedment and microtomy among other things.

Recent important innovations in and evaluations of preparatory procedures have, however, come to ease the above stated problem. These include works on fixatives (Kalt and Tandler, 1971), fixation (Zalokar, 1971), microtomy (Glauert and Phillips, 1965), embedment (Coulter, 1967), instrumentation and methodology (Beer, 1965; Boyde and Wood, 1969; Mahowald and Turner, 1978).

With these latter developments in mind, it was, therefore, the intention in this investigation to examine the embryonic development of the stable fly from the time of oviposition to when the first instar larva hatches from the egg, using principally the scanning electron microscope (SEM) supplemented by transmission electron and light microscopes. The specific period chosen for investigation is particularly important in studies involving chemical and biological insect control agents with ovicidal activity.

SYNOPSIS OF THE LITERATURE ON INSECT EGG AND EMBRYONIC DEVELOPMENT WITH PARTICULAR REFERENCE TO THE STABLE FLY AND CLOSELY RELATED DIPTERA

Although specific information about embryogenesis in this fly is sparse, a more complete picture or insight may be obtained by considering extant literature on closely related Dipterans and collating any relevant information of embryologic features characteristic of class Insecta.

Egg Structure. Insect eggs occur in a variety of sizes and forms. The common housefly eggs, for instance, measure only one millimeter in length whilst some Acrididae eggs may be 8 mm long and 1 mm in diameter. They may be banana-shaped as in stable fly or conical as in moths, among other shapes.

The embryo typically contains a mass of yolk. This latter is ramified by a reticulum of cytoplasmic material that in addition surrounds the egg nucleus and forms an outer bounding layer called the periplasm. A thin vitelline membrane, lying internal to the egg shell, typically surrounds the whole embryo.

Variations occur in this generalized picture. Thus a periplasm may in Aedes vexans and at oviposition, be grossly indistinct (Telford, 1957) or lacking (Horsfall et al., 1973). Telford (1957) also noted the absence of the vitelline membrane until 24 hours post-oviposition in Aedes vexans although others (like Quattropani and Anderson, 1969) hold that this membrane is typically formed by follicle cells during oogenesis. Moreover Anopheles maculipenis according to Nicholson (1921) and Aedes

vexans according to Horsfall et al., (1973) notably lack a distinct egg nucleus at oviposition.

The shell or chorion is largely a product of follicle cells during oogenesis (Beament, 1946; King and Koch, 1963). Imprints of these follicle cells are often left on the outer surface of shells as reticulate markings (Beament, 1946). Sometimes, as has been seen in Rhodnius, Carauscius and Drosophila, two distinct layers are recognizable in the chorion or shell--an outer exochorion and an inner tanned endochorion (Wigglesworth and Beamont, 1950; Turner and Mahowald, 1976). At other times no clear distinction could be made. Most terrestial insects, however, have chorionic mesh works that hold gases. Musca has a complement of two such air spaces--one outer and one inner to the main mass of chorionic material (Hinton, 1967). Further, some chorionic features are not generalized. For instance in some Diptera, e.g. Calliphora (Hinton 1960), two longitudinally running 'hatching' lines are present. These usually represent lines of structural 'weakness' and serve for the exit of the larva at hatch. In other eggs, the chorion may possess a cap or operculum, as in Drosophila whose eggs also possess extra-chorionic appendages (Turner and Mahowald, 1976).

Micropyles exist as canals through which the sperm may gain entry into the egg (Nonindez, 1920). Species complements vary. Dipteran eggs usually have one, while a complement of up to 40 may be met in Locusta, (Roonwal, 1954). In Oncoleptus these micropyles are borne on chorionic stalks (Southwood, 1956).

More specifically, Newstead (1906) noted that the eggs of the stable fly are about one millimeter in length, coriaceous, elongate, curved on one side and grooved on the other with shells possessing surface polygonal reticulate markings.

Oviposition. Characteristically blood sucking Diptera and some other vectors, engage in blood feeding in the adult stage for the purpose of parity or egg development. However, a genetically based, hormone-controlled condition--autogeny--which for maximum expression requires ideal nutritional and environmental conditions, may occur whereby part of a vector population can develop a first batch of eggs without a blood meal (Clements, 1963). In the stable fly, although Hindle (1914) claimed autogeny does occur on occasions, most workers now agree that two or more blood meals are necessary before egg-laying commences. Bishop (1913) went further to state that more blood meals are needed as environmental temperature falls.

On the topic of suitability of ovipositional sites, there exists great variability in choice among insect species, the option being determined presumably by the need to provide a protective site environment which in addition should be as close as possible to the food preferences of the hatching larva. Thus Musca lays its eggs in fermenting moist manure or excrement whilst the screw worm-fly prefers edges of wounds on animals. According to Bishop (1913) and Hindle (1914) stable fly eggs are preferably laid below surface of decaying or soggy vegetable (e.g. hay or grain) matter and less so in animal manures.

With regards to the stable fly's habits, the literature on the number of eggs per oviposition, oviposition times per entire adult female life, total number of eggs per all ovipositions as well as time of commencement and period of life for egg-laying appeared to contain no hard and fast rules or pattern. Thus Bishop (1913) recorded three "greatest" number of oviposition per lifetime yielding a total of 273 eggs whilst his contemporary Mitzmain (1913) had 20 ovipositions for a yield of between 632 and 820 eggs. Parrs (1962) recorded an average of 300-400 eggs per female fly in 10-12 layings and Hoffman (1968) obtained a maximum 307 eggs per female in laboratory-reared specimens. On the other hand, the six to eight day period after emergence of the fly appeared to cover most observations regarding the beginning of oviposition; according to Berry and Kunz (1978) who, in addition, concluded from their own observations that oviposition was unaffected by humidity but greater and began at younger ages with higher temperatures. Also Hoffman (1968) noted that oviposition decreases and becomes erratic with age.

Embryonic Development. Early publications on Dipteran embryology included such works as those by Hardenburg (1929), Gambrell (1933), Butt (1934), Lassman (1936) and Poulson (1937). In more recent times there have been others from Sonnenblick (1950, Poulson (1950), Ede and Counce (1956), Anderson (1962, 1963, 1964, 1966), Schoeller (1964), Craig (1967), Davis (1967), Wright, T. (1970), Zalokar and Erk (1976), Cantwell et al., (1976), Ueda and Okada (1977), Fullilove and Jacobson (1971, 1978) and Mahowald and Turner (1978).

Cleavage and Blastoderm Formation. Cleavage in Dipteran embryos commences soon after oviposition, being possibly preceded only by processes involved in the maturation of the egg, fusion of the pronuclei and formation of the zygote nucleus. In Dacus tryoni the latter events are confined, notably, to the first half hour post-oviposition (Anderson, 1962). In Drosophila melanogaster it may further be noted, these post-ovipositional pre-cleavage events are obligatorily completed whether or not the egg is fertilized (Doane 1960).

In general, cleavage rates tend to be species specific, slower among the Nematocera than Cyclorrhapha, and decrease with elapsed time. Thus at 25° C, an average of one cleavage every 10 minutes has been recorded for Drosophila melanogaster (Sonnenblick, 1950), 15 minutes for Dacus tryoni, (Anderson, 1962), eight minutes for Musca domestica (Cantwell et al., 1976 and six minutes for Cocchliomyia hominivorax (Reiman, 1965) whereas Christopher (1960) obtained an average of 20 minutes for the Nematoceran Culex molestus at 18° C.

Anderson (1966) reviewed 30 publications to conclude that typically cleavage in the Diptera is centrolecithal and proceeds by rapid synchronous nuclear divisions though he conceded that factors maintaining the synchrony of mitosis were (and are still) not understood.

At the inception of the blastoderm, the bulk of the cleavage nuclei migrate into the periplasm. The cleavage stage which precedes this event appears to vary with species (Anderson 1962).

Thus migration of nuclei in D. melanogaster follows the ninth cleavage stage (Turner and Mahowald, 1976), tenth in C. molestus (Christopher, 1960) and seventh in Sciara coprophila (DuBois, 1932). Again, the number of nuclei that invade the periplasm (since some are typically left behind in the yolk matrix, see below) appears not to be a definite figure. For instance, Turner and Mahowald (1976) using scanning electron microscope techniques obtained a range of figures -373-409- for nuclei invading the periplasm in five D. melanogaster embryos after the ninth cleavage.

Vitellophages. Those nuclei that fail to migrate to the surface eventually form primary vitellophages; often after some mitosis not in synchrony with the rest of cleavage nuclei. Thus in D. tryoni, 38 nuclei left behind after the seventh cleavage divided three or four times to yield 300-600 primary vitellophages (Anderson, 1962) whilst approximately 50 of the possible 256 nuclei produced at the sixth cleavage stage in D. melanogaster were involved in this process and subsequently underwent a single synchronous division to yield 100 definitive primary vitellophages (Sonnenblick, 1950). Cantwell et al., (1976) gave the range 150-200 for remaining nuclei in Musca domestica after 80 minutes of development.

Secondary vitellophages are known in the literature to form from cells that migrate back into the yolk matrix (before cell boundaries formation). They have been reported, amongst others, in Aedes vexans (Horsfall et al., 1973), Culex fatigans (Davis,

1967) and Dacus tryoni (Anderson, 1962). Tertiary vitellophages are also known. Davis (1967) reported them derived from the midgut rudiments in Lucilia sericata during gastrulation. It is worthy of note that Horsfall et al., (1973) observed that yolk cells do not divide in synchrony with cleavage nuclei nor were they ever observed in mitosis.

Pole Cells. Dipterans characteristically possess pole cells which incorporate the posterior pole plasm, polar granules and yolk spheres. They form from cleavage nuclei that directly invade this polar region. They typically lose mitotic synchrony with and are thus never part of the blastoderm; (Horsfall et al., 1973, Mahowald and Turner, 1978). Sometimes a specific number of nuclei--e.g. four for D. tryoni (Anderson, 1962) and 12 for Melophagus ovinus (Lassman, 1936)--regularly invade the polar region in a species, but in the greater number of cases studied, this number remains a range.

Moreover, the time of invasion and the number of pole cells finally obtained after mitosis appear to vary among species. Thus Mahowald and Turner (1976) recorded the invasion as following ninth cleavage and the final number of posterior pole cells as between 23 and 52 (average 37) from an initial 10-20 cells in D. melanogaster. Further there are more pole cells per species in the Cyclorrhapha than the Nematocera (Horsfall et al., 1973).

Cytokinesis, which comes as the final stage of cellular blastoderm formation, is usually preceded by a relocation of yolk constituent of the embryo to the central area of the egg

accompanied by formation of a syncytial yolk sac and the concentration of cytoplasmic organelles at the periphery (Sonnenblick, 1950; Mahowald, 1963 a and b; Turner and Mahowald, 1976). In addition, blastoderm nuclei undergo species specific rounds of synchronous divisions before cytokinesis; which increases the final number of nuclei in the blastoderm. In this respect there are four such rounds (extra to the previous nine) in D. melanogaster (Zalokar and Erk, 1976) and three in C. molestus (Christopher, 1960).

Finally the time taken to complete blastoderm formation varies with experimental factors and species. Cantwell et al., (1976) had 2 1/2 hours for the housefly at 26.5^{o} whilst similar data for D. tryoni were 7 1/2 hours at 25^{o} C, (Anderson, 1962) and for C. hominovarax, 2 1/2 hours at 25^{o} C, (Reimann, 1965). Gastrulation. From Poulson (1950), Anderson (1962), Schoeller (1964), Davis (1967) to Horsfall et al. (1973), Cantwell et al. (1976) and Mahowald and Turner (1978) along with older works mentioned earlier, the classical generalized steps of gastrulation in the Diptera may be followed.

Typically gastrulation is initiated by the formation of the ventral furrow; the groove formed when the broad plate of presumptive mesoderm folds into the interior along the ventral midline. This furrow eventually closes off as either a solid or hollow rod of cells depending on the species, Horsfall et al., (1973). It is worth of note that this mode of mesoderm internalization (by invagination) contrasts markedly with that obtaining

in some other insects like the Hymenoptera (e.g. in Habrobracon junglandis; Amy, 1961) in which the broad plate sinks rather than invaginates. Moreover, an idea of the "broadness" of the presumptive mesodermal plate could be obtained from Mahowald and Turner (1978) who calculated that 15-18% of all blastoderm cells invaginate in the first 15 minutes of gastrulation in D. melanogaster. Subsequently the lips of presumptive ectoderm along this invagination join over the mesodermal rod thereby internalizing it. These cellular migrations thus give rise to a ventral germ band comprising presumptive ectoderm and mesoderm.

Shortly after the inception of the ventral furrow two endodermal rudiments--the anterior and posterior mid-gut rudiments--appear on the ventral midline a little on the anterior and posterior ends of the ventral furrow respectively. Turner and Mahowald (1977) noted with SEM that although they bear the same bulbous projections as the ventral furrow, they persist longer before being internalized and may bear distinctly long microfibrils on their free surfaces rather than adjacent portions of the ventral furrow.

In addition and with the ventral furrow closing over the mesodermal rod and mid-gut rudiments, two other rudiments appear on the ventral mid line. One, the stomodeal rudiment, is found a little anterior to the locations of anterior mid-gut invagination, the other, the proctodeal rudiment, is positioned a little anterior and ventral to the posterior pole cells. The stomodeal rudiment commences invagination soon after formation but the

invagination of the proctodeal rudiment is delayed until late in the subsequent process of germ band elongation. The stomodeum eventually receives the internalized anterior mid-gut rudiment on its internal end whilst the proctodeal invagination eventually receives the posterior mid gut rudiment.

In Diptera, the elongation of the germ band around the posterior tip of the embryo and then cephalad have been consistently described in the literature. On the other hand, only in a few instances, as in Simulium pinctipes (Gambrell, 1933), Sciara coprophila (Butt, 1934) and Glossina tachnoides (Hagan, 1951) have there been descriptions of (limited) elongations of the anterior end of the germ band over the anterior pole. In disagreement with Anderson (1966), Turner and Mahowald (1977) noted that while the presumptive mesoderm of D. melanogaster participates in the initial posterior end elongation it normally stops short of the posterior tip--spreading eventually from its ventral location to underlie the ectoderm.

Another characteristic feature of Dipteran embryology is the presence of temporary transverse folds that accompany gastrulation and appear simultaneous with the elongation of the germ band. (Aedes vexans is an exception in that it exhibits no furrows, Horsfall et al., 1973). The pattern and number of these furrows differ with the species; with the only exception of the cephalic furrow which is constant in Cyclorrhapha, (Anderson, 1962). Typically the cephalic furrow is found to mark off circumferentially the anterior one third of the embryo and has been shown

to be formed in D. melanogaster (Turner and Mahowald, 1977) by the contraction of a single row of cells which then draws inward the apical surfaces of adjacent cells.

With regard to the other folds, Guichard (1971), quoted by Horsfall et al., (1973), described seven furrows posterior to the cephalic furrow in Culex pipiens, but there were just five in D. tryoni (Anderson, 1962) and only two in D. melanogaster (Turner and Mahowald, 1977).

There is also a divergence in the interpretation of the significance of these furrows by various workers. Cantwell et al. (1976), for instance, felt that the furrows in Musca merely reflect the tensions produced by the ventral and cephalic furrows. Mahowald and Turner (1978) supported the view that they are reflective of metameric regions of the embryo. Guichard (1971) believed only the cephalic furrow have a chance of having connection with metamerism. It may be noted, however, that all other furrows always appear posterior to the cephalic furrow and in addition disappear before it and ahead of the elongating germ band (Anderson 1962).

Another consistently observed feature of Dipteran gastrulation is that the embryonic ectoderm lateral and anterior to the mid-ventral rudiments always remain unchanged from their columnar blastoderm form throughout gastrulation, except for the formation of neuroblasts in late gastrulation and possible mitotic replacement of migrating posterior pole cells (Turner and Mahowald, 1977; Anderson, 1962).

On the other hand, the extraembryonic ectoderm differentiates by attenuation and spreading of its cells as a dorsal epithelium. It then comes to lie between the embryonic ectoderm anteriorly and laterally and the proctodeal wall posteriorly. The subsequent elongation of the germ band then tends to push this layer of cells ahead of and lateral to it. The layer in Cyclorrhapha is usually rudimentary and resorbed in late segmentation or at dorsal closure (Turner and Mahowald, 1977; Anderson, 1966).

Pole Cells. Two pathways exist for the eventual migration of posterior pole cells into the interior of the embryo. According to Anderson (1966) in the Nematocera (except the Culicidae) the cells migrate before the onset of gastrulation into the embryo and during gastrulation move through the yolk, in two groups which come to rest on either side of the ingrowing posterior mid-gut/proctodeal rudiments. On the other hand, in Culicids and the Cyclorrhapha, the cells sink into the migrating posterior mid-gut rudiment, as this move anteriorly, from where they come to lie either in the hemocoel or the wall of the posterior mid-gut/proctodeal rudiments.

It is noteworthy that all of the pole cells in Nematocera receive germ-cell determining ooplasmic material and develop as germ cells. However, in the Cyclorrhapha only a proportion have this potential; the remainder, variously in different species, acting as vitellophages or being absorbed into the gut wall (Anderson, 1966).

Regulations Centers. In general, formation of the germ band is regulated by two centers, a posterior activating center that triggers and a (second) differentiation center located usually in the prothorax and operating during blastoderm formation (Anderson, 1961; Davis, 1967). The activity of the differentiation center is later taken over by segmental centers when segmentation is completed. Notably in D. melanogaster, Ede and Counce (1956) found cinematographically that a yolk contraction in the vicinity of prothorax preceded the onset of gastrulation. Yao (1950) also noticed that histodifferentiation spreads from a thoracic focus in the same insect embryo.

Organogeny. Postgastrulation developments usually involve histodifferentiation and morphogenesis of presumptive rudiments that have been relocated by the process of gastrulation. In its early stages and as it affects the embryo as a whole, organogeny manifests as segmentation, contraction of the germ band, dorsal closure and, in the Cyclorrhapha, involution of the head (Anderson, 1966).

Morphogenesis. Following gastrulation the process of segmentation commences, proceeding from the anterior end posteriorly. In the higher Diptera, since the premandibular and antennal segment are not clearly depicted, the first obvious segments are the post-orals, mandibular, maxillary and the labial (Mahowald and Turner, 1978). All segments are delineated by infolding of developing epidermis which originate from the presumptive ectoderm (Poulson, 1950).

Segmentation of the mesoderm sometimes occurs with somite formation following ectodermal segmentation and with each somite giving rise to somatic and splanchnic components; (Butt, 1934; DuBois, 1932). In some other cases, splanchnic mesoderm may show no segmentation (Davis, 1967). It is noteworthy that splanchnic mesoderm gives rise to gut musculature (Anderson, 1962) and also to the fat body, hemocytes and gonad sheaths.

A shortening of the germ band accompanies segmentation and results in the retreat of the proctodeal opening to its definitive posterior ventral position. In addition, the dorsal closure which involves the upgrowth of the dorsolateral edges of the segments, commences from the anterior end posteriorly.

In Musca domestica Cantwell et al. (1976) observed that organogenesis up to the end of dorsal closure and segmentation may be achieved by seventh hour post-oviposition at 25° C whereas in Dacus tryoni the same stage is reached at about 24 to 28 hours under similar experimental conditions.

In Cyclorrhapha, the process of the involution of the head commences after the dorsal closure has been completed anteriorly and the shortening of the germ band is continuing (Poulson, 1950; Ludwig, 1949; Ede and Counce, 1956; Butt, 1960; Mahowald and Turner, 1978). It involves, in higher Diptera, a migration of head ectoderm into the stomodeum to form the lining of the pharynx and is accompanied by an anterior migration of the post oral segments to their definitive position vis-a-vis the oral cavity.

Histodifferentiation. In general a composite picture of histodifferentiation may be obtained from a host of authors. These include DuBois (1932), Butt (1934, 1960), Auten (1934), Gambrell (1933), Bull (1952), Poulson (1950), Ede and Counce (1956), Anderson (1962, 1966), Davis (1967), Schoeller (1964), Horsfall et al., (1973) and Cantwell et al., (1976).

In the development of the gut, the stomodeum eventually produces the oesophagus and central core of the proventriculus. The anterior and posterior mid-gut rudiments produce strands that unite to enclose the yolk and form the mid-gut. The proctodeum gives rise to the hind-gut. Malphigian tubules develop usually as outgrowths of the distal end of proctodeum or sometimes from the mid-gut (Poulson, 1950). In Cyclorrhapha, salivary glands derive from labial segments whereas in Sciara coprophila they originate from stomodeum (DuBois, 1932). Stomodeum, in Cyclorrhapha, may also give rise to the stomatogastric ganglion (Poulson, 1950, Anderson, 1962, Davis, 1967).

The tracheal system develops from presumptive rudiments that are segmentally arranged and paired. These rudiments grow inwards, bifurcate and unite to form branches and trunks.

The central nervous system as noted previously develops from paired presumptive neuroblast groups that differentiate from embryonic ectoderm. According to Poulson (1950) they enlarge and undergo a series of unequal cell division after separating from the ectoderm. They eventually give rise to the brain and ventral nerve cord.

MATERIALS AND METHODS

Fly Rearing and Egg Collection. The strain of stable fly employed in this study was obtained as pupae from Texas A & M University, College Station, Texas. Adult flies were raised in wire cages in a rearing room maintained routinely at 80° F and 70% relative humidity on a 17-hour/day photoperiod. A meal of citrated bovine blood on a swab of cotton wool was provided daily, the flies being allowed to feed freely.

For egg collection flies oviposited on wet black muslin (wrapped around a wad of cotton wool) placed on the cages. For each experimental session (i.e., oviposition to hatching of first instar larva) only eggs collected over a precise 10-minute period were used. These were spread on water-soaked, two-ply filter papers in petri dishes at the rate of 200-300 eggs per dish. Finally the dishes were wrapped in black muslin and incubated in a rearing chamber at 30° C.

Egg Sampling and Dechorionation. A ten-egg sample was taken for each stage of development examined. Assuming the middle of ovipositional period as zero hour, samples were initially dechorionated and fixed very 30 minutes for the first five and a half hours, every hour for the next six hours and subsequently every three hours until hatch. In addition, a sample designated stage 0 was fixed at 15 minutes post (i.e. middle) oviposition. Later still other collection times were introduced as necessary.

All specimens were dechorionated by immersion in 2-3 ml 25% Durox R* (active principle 5.25% sodium hypochlorite) for three

minutes. They were then rinsed three times in distilled water before any further treatment.

Preparation of Specimen for Light Microscopy (LM). To ensure rapid and uniform penetration of the embryo by fixative and prevent distortion of surface features by compression, the vitelline membrane enveloping the embryo was removed by appropriate modifications of a procedure previously described by Zalokar (1971) and improved on by Mahowald and Turner (1978). This procedure involved the immersion and vigorous shaking of the embryos in heptane mixed with equal volume of 50% acetic acid for one minute. The embryos were then floated on to a fixative--which was a modified Carnoy's preparation containing six parts isopropanol, three parts chloroform and one part of glacial acetic acid. After the heptane layer had evaporated the embryos were submerged, and with the aid of sharpened tungsten wires, their vitelline membrane was removed. Fixed embryos (three hours fixation time) were embedded in paraffin, serially sectioned at 6u and stained by regressive hematoxylin and eosin procedure (Appendix 1). Some specimens were dechorionated and placed in hanging drop preparations and photographed by indirect (transmitted) light technique with the aid of an Olympus Universal Research Microscope Model Vanox.

Preparation of Specimen for Scanning Electron Microscopy (SEM). The egg dechorionation and vitelline membrane removal procedures were as described above (for light microscopy) except that (i) 50% biological grade glutaraldehyde was mixed with heptane in place of acetic acid and (ii) the fixative used was a

trialdehyde (3% glutaraldehyde, 2% formaldehyde, 1% acrolein and 2.5% dimethyl sulfoxide), Kalt and Tandler (1971).

As previously described by Mahowald and Turner (1978) embryos' internal structures were exposed with sharpened tungsten wires while still in the fixatives, followed by sonification for one minute aimed at removing loose fragments from the specimens.

Embryos were allowed to fix overnight, subsequently given three brief rinses in a sucrose-cacodylate buffer, (pH 7.2) and post-fixed in 1% osmium tetroxide in 0.1 M cacodylate buffer for one hour. Three 15-minute rinses in water followed before their dehydration through a graded series of ethanols (30, 50, 70, 95, 100%) at ten minutes per step.

Using CO_2 and absolute ethanol as intermediate fluid the embryos were dried by critical point drying technique and afterwards mounted on stubs with the aid of double sticker tapes. They were coated in a KSE-2AM evaporator with a thin layer of carbon followed by gold-palladium and photographed with an ETEX autoscan Model U-1 Scanning Electron Microscope (Appendix II).

Preparation of Specimen for Transmission Electron Microscopy (TEM)

Procedures for egg collection, dechorionation and devitellination as well as embryo fixation and dehydration were as described above for the SEM.

After dehydration embryos were gradually infiltrated with Spurr's epoxy resin through a number of absolute alcohol/Spurr's resin mixtures as follows:

100% alcohol: Spurr's 2:1 for two hours

1:1 for two hours

1:2 for two hours

100% Spurr's for three hours

Specimens were finally embedded in freshly prepared Spurr's resin at 70° C for eight hours in flat embedding molds. However, because of the need to section embryos during microtomy in specific orientations, the resultant casts were cut up with a saw and mounted (with a Duco-cement[R***] glue) on plastic bases according to the desired orientation.

Gold and silver colored sections, indicating section thickness of 0.060-0.150u were cut with glass knives on a Reichert OM-2 ultramicrotome and mounted on carbon-coated, Formvar[R**]-filmed 100-mesh copper grids. These were finally stained with uranyl acetate, counterstained with lead citrate and photographed with a Phillips 201 electron microscope at 60KV (Appendices II and III).

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*Durox[R] is a trademark of Mid America Chemicals, Phoma, Oklahoma.

**Formvar[R] is a trademark of Ladd Research Industries, Inc. Burlington, Vermont.

***Duco-cement[R] is a trademark of E. I. duPont de Nemours and Company, Inc., Wilmington, Delaware.

RESULTS

For descriptive purposes and with Stage 0 denoting the time previously ascribed to it (i.e. 15 minutes post oviposition), all stages described in this report are numbered for every half hour post-oviposition until hatch. Thus stage 1 denotes 30 minutes post-oviposition; stage 4, two hours post-oviposition; stage 9, 4 1/2 hours and so on.

EGG STRUCTURE

Under the rearing conditions described, the stable flies started mating as early as the sixtieth hour post-ecdysis, with egg-laying commencing about the sixth day and lasting up to the twentieth day. Generally flies oviposited in heaps of six to 20 eggs if left undisturbed, the posterior end of each egg emerging first. Peak oviposition almost invariably fell between the eighth and eleventh day.

Each egg, at oviposition, is white, elongate and measures 950-1250u (length) by 200-280u (diameter). It is slightly curved along its anteroposterior axis, grooved on the concave (dorsal) side and has a blunt anterior pole which is decidedly narrower than its posterior (Plate 1, Fig. 1). The external surface of the shell, which eventually darkens to a dirty white, is sculptured; a random pattern of penta--and hexagonal plates with admixtures of triangular and quadrangular plates at the poles. Plate sides vary between 2-16u in length (Plate 1, Figs. 1, 2 and 3). Two longitudinal lines--the hatching lines of early

authors--joined as arcs about 15u from either pole to encompass the dorsal groove (Plate 1, Figs. 2 and 3). They also, in effect, demarcate the lateral extents of the dorsal shell plate (Plate 1, Fig. 6, see below). A micropyle, with an average 4u diameter opening, is located two or three rows of "shell" plates ventral to the anterior arc of the hatching lines (Plate 1, Fig. 3). The shell bears no appendages.

At the hatching lines, sections of the egg shell (Plate 1, Figs. 4 and 5), revealed an outermost network of chorionic material that was 0.12-0.16u thick. A subjacent space, about 0.5u thick, then follows which is transversed by narrow tipped struts or bosses. The resultant interstices are about a micron wide. The struts originate from a middle chorionic layer which, at 3.5-4.0u width, is proportionately the widest part of the shell. This part possesses aeropyles that are continuous with a second airspace immediately on the inner side of this layer; just as they are continuous with the outer airspace. The innermost layer of the shell is another chorionic network like the outermost grid.

Grossly and in cross section, the shell can be considered composed of two segments: (i) a semicircular plate, half as thick as the hatching line format, that accounts for the two lateral and convex sides of the egg and (ii) a thickened dorsal plate whose thickness decreases towards the posterior from the anterior end (Plate 1, Fig. 6). Lines of union of the two plates (which thus constitute the hatching lines mentioned above) revealed an interdigitating arrangement of lamellate extensions of chorionic material from adjacent plate edges (Plate 2, Fig. 7).

A thin, transparent, glistening vitelline membrane is present immediately within the chorion (Plate 2, Figs. 8 and 9). It is closely associated with the surface of the embryo around the latter's mid-section but becomes reflected out towards the chorion at the poles (Plate 2, Fig. 10). It also possesses a micropylar opening at the anterior pole in conjunction with the chorion.

Scanning electron micrographs (SEM) of the surface of the embryo at stages 0, 1 and 2 showed a coverage of thin microprojections (Plate 2, Fig. 11). Averaging about a micron in height, many of these projections appeared filiform whilst others bear bulbous ends. Transmission electron micrographs (TEM) of the same region revealed these as mere extensions of the periplasm--the thin layer of cytoplasm that marks the periphery of the embryo's cytoplasm (Plate 2, Fig. 12). At oviposition, this periplasm is a few microns thick and is singularly devoid of vacuoles and yolk spheres characteristic of the rest of the cytoplasm. It is, however, appreciably extensive at the poles and may contain granules especially at the posterior pole.

The outer periplasmic layer is continuous with the central cytoplasmic reticulum of the embryo which later encloses a large number of vacuoles and yolk spheres typically encountered in these embryos. The vacuoles vary in their sizes from the sub-microscopic to some of 0.5u diameter. Some yolk spheres measure up to 3u in diameter (Plate 2, Fig. 13). The zygote nucleus, surrounded by a cytoplasmic halo, initially lies towards the midline in the anterior half of the embryo but daughter nuclei

are to be found in locations described below as cleavage proceeds. The polar nuclei degenerate as cleavage proceeds.

CLEAVAGE AND FORMATION OF THE BLASTODERM

In the period before the acellular or syncytial blastoderm was formed, the thin, initial periplasmic layer increased gradually in thickness. This was accompanied by increased vacuolation of the cytoplasmic reticulum and concentration of the yolk spheres longitudinally around the center of the embryo (Plate 2, Fig. 14).

Meiotic figures were not observed at any stage including stage 0. However, cleaving nuclei were readily followed afterwards especially as they showed good reaction to chromatin stains during cleavage (Plate 3, Fig. 15).

Initially cleavage, without cytokinesis, occurred at an average rate of once every eight minutes, but this slowed down considerably towards the end of cleavage to about once every 15 minutes. The cleavage proceeded essentially as synchronous nuclear divisions. Early cleavages resulted in an aggregate of nuclei which, commencing at about the fourth or fifth cleavage session, started migrating through the embryo, especially posteriorly. This move allowed the distribution of the nuclei in a circular fashion around the midline (Plate 3, Fig. 16) as well as effecting a longitudinal dispersal throughout the embryo (Plate 3, Fig. 17).

The rearrangement was followed at about the ninth cleavage stage by movement of the bulk of the cleavage nuclei towards the periplasm from their circumcenter location. This later move left behind a small mass of nuclei--120 $\pm$ 20 --in number whose eventual fate are described below (Plate 3, Fig. 18). On the other hand, the arrival of the migrating nuclei at their final periplasmic locations were at individual rates rather than as a massed synchronised event (Plate 3, Fig. 19).

SEM studies of changes in the embryo's external surface, at this time of blastoderm inception, showed that this invasion of the periplasm resulted in the development of bulges on the free surface. The bulges were still covered by the previously described microprojections but each reflected the presence of an underlying nucleus. Internally, a syncytial arrangement thus existed in which cellular partitions have not appeared between the nuclei. This was then the stage of the syncytial blastoderm (Plate 3, Fig. 19).

After the formation of this acellular blastoderm, cleavage rounds were monitored by increases in number and pattern of these external bulges. It was observed, for instance, that cleavage rounds commenced at the equatorial region of the embryo and proceeded in sequence polewards (Plate 3, Figs. 20 to 22). Also, four more cleavage rounds took place before delimiting cell furrows started to appear between the nuclei internally, thus indicating the onset of the cellular blastoderm (Plate 3, Fig. 23).

Meanwhile and about the time of periplasmic invasion by cleavage nuclei, the granular posterior pole plasm was invaded by 9 ± 1 nuclei (resulting in the appearance of characteristic surface bulges) (Plate 4, Fig. 24). Eventually, this group of bulges, after about two asynchronous divisions that yielded 36 ± 2 cells or bulges, formed a distinct cap of cells on the posterior pole (Plate 4, Figs. 25 and 26). These posterior pole cells (PPC) formed bulges that were individually conspicuously larger than other bulges hitherto observed, although, at this stage, they were all bearing grossly similar surface microprojections (Plate 4, Fig. 26).

Centrally within the embryo, a syncytial yolk sac gradually became evident from stage 4 simultaneously with the process of cellular delineation occurring in the periplasm. Within its confines were found, initially, the clumps of nuclei mentioned previously as remnants of the mass migration to the periplasm (Plate 4, Fig. 27). These nuclei, referred to as primary vitellophages, were small, dark-staining masses surrounded by thin cytoplasm (Plate 4, Fig. 27). Later they underwent asynchronous divisions to increase their number in the period before the cellular blastoderm was completed and were eventually dispersed through the yolk substance. In some sections, as in Plate 4, Fig. 28, they increased in number through a process of nuclear reinvasion from the poles--secondary vitellophages.

A complete cellular blastoderm (Plate 4, Fig. 29) was usually evident at stage 5 or early stage 6. The actual

cytokinetic process of cellular partioning of the syncytial blastoderm took an average of 27 minutes to complete and invariably came after the thirteenth cleavage stage.

GASTRULATION AND SEGMENTATION

At the onset of gastrulation, a fractured specimen, under SEM, showed a large yolk sac filled with variously sized yolk spheroids within the blastoderm. Placed more or less centrally within the embryo (in cross section), each sac was delimited by a perilecithal cytoplasmic sac and averaged a diameter of 70u (Plate 4, Fig. 29). The blastoderm cells were tall columnar types with average width of 6.5u. Individual cell heights, however, decreased around the embryo dorsad; from the center cells possessing a mean height of 50u to about 36u cell-lengths on the dorsum. Externally the cellular apices still retained their bulging forms although their surface microprojections appeared less prominent, thus making the embryonic outer surface smoother than hitherto.

Ventral Folds (Plate 5, Figs. 30-33). Stage 6 embryo showed the first external evidence of germ band formation. This appeared in the form of a gradual infolding of cells--the ventral furrow (VF)--along the midline of the ventral surface of the embryo (Plate 5, Fig. 30). This early cellular movement extended lengthwise through approximately the middle three-fifths of the embryo initially and internally tended to at first flatten and later push into the ventral perimeter of the yolk sac. The

infolding produced a rod of (presumptive mesodermal) cells over which the ventral ectoderm eventually closed (Plate 5, Figs. 31 and 32).

Two other ventral surface centers of cellular movements were also evident in slightly older specimens. One of these, the anterior midgut rudiment (AMR), appeared on the midline like the VF but a little beyond the point of intersection of the VF and the cephalic furrow (see below). The other, the posterior midgut rudiment (PMR) occupied a position ventral and anterior to the PPC. This later fold subsequently assumed a pocketlike shape during the elongation of the germ band (see below) into which the migrating PPC were received and carried anteriorward (Plate 5, Fig. 32).

Both infoldings (Plate 5, Fig. 33) constituted early endodermal germ band formations and were subsequently internalized. In this respect the anterior midgut rudiment (stages 6-7) came to lie internal and attached to an anterior ectodermal infolding--the stomodeum--which developed late on the midventral surface a little anterior to the AMR position. On the other band and prior to the ultimate anterior elongation of the germ band, the PMR came to lie internal and attached to a posterior ectodermal invagination--the proctodeum. The last eventually gave rise to the terminal portions of the embryonic gut.

Dorsal Folds (Plate 5, Figs. 34 and 35; Plate 6, Figs. 36 and 37). During stage 6 and at about one third of the embryo length from the anterior pole, a thin line of invaginating cells--the cephalic

furrow--appeared on the lateral and dorsal aspects of the embryo (Plate 5, Fig. 34). Within the stage it described a ringlike path by extending obliquely forward and around the ventral anterior end of the embryo. In late stage 6 and early 7, the furrow deepened steeply all around the embryo to a point where the cephalic portion of the embryo was almost cut off (Plate 6, Fig. 37).

Starting in late stage 6 and through most of stage 7, five major transversely running (none ever touch the VF) infoldings appeared together between the deepening CF and the advancing PMR (Plate 5, Figs. 34 and 35; Plate 6, Fig. 36). These lines were transistory and lasted only an average of 40 minutes. Among the first folds to appear were the anterior and posterior transverse folds. The central transverse fold, usually occupying a middle position on the embryonic equator, was flanked by the obliquely running antero-central and posterior-central folds. The folds disappeared in sequence from the posterior to the anterior end.

Formation of the Amnioserosa and the Elongation of the Germ Band. After the formation of the mesodermal rod of cells (vide supra), there was an initial joint extension of the germ band (ectoderm and mesoderm) posteriad which thus pushed the PMR dorsad and anteriad. The ectoderm alone then continued the elongation around the posterior embryonic tip and anteriorly; with the mesoderm cells subsequently spreading to underlie the ectoderm from their ventral position (Plate 5, Figs. 31 and 32). As

mentioned previously, the PPC finally came to lie in a vesiculated depression formed by the PMR and were carried cephalad by the advance of the latter (Plate 5, Fig. 32; Plate 6, Fig. 39).

In late stage 7 embryo, the transverse folds individually disappeared ahead of the advancing PMR. Simultaneous with their disappearance and in the region they had occupied, a thin sheet of cells was formed (posterior to the CF but ahead and laterad of the PMR). This was the amnio-serosal layer (Plate 6, Fig. 38). As a cellular sheet, it was loosely attached to the yolk sac membrane and very often peeled off it easily (especially with any relatively vigorous sonication) (Plate 6, Fig. 40). It gradually disappeared with the advance of the germ band although some portions were retained well into the period of segmentation (Plate 6, Figs. 38 and 39).

The ultimate elongation of the germ band brought the PMR contiguous with the cephalic furrow (CF). At this time the PMR became internalized and attached to the newly formed proctodeal opening (vide supra) (Plate 5, Fig. 33).

Segmentation. Dorsally and with germ band elongation completed the process of segmentation was externally apparent, before the disappearance of the CF and the amnio-serosa, as periodic lines of invagination projecting laterad of the mesectodermal band (Plate 6, Fig. 39; Plate 7, Figs. 41 and 42). On the ventral surface, these were clearly evident in the region of the embryo posterior to the CF dorsally and to the stomodeal invagination ventrally (Plate 6, Fig. 40). Studies using indirect light

microscopy revealed that at the time of retreat of the germ band, there was a corresponding segmental distribution of mesoderm internally (Plate 7, Fig. 43).

In late gastrulation, tracheal pits appeared on the lateral aspects of each segment; there were ten in all corresponding to the last two thoracic segments and first eight abdominal segments (Plate 7, Fig. 44). During the same period and with the stomodeum deepening, rudiments of three cephalic appendages were also becoming apparent. These were, from the anterior, the mandibular, maxillary and labial rudiments (Plate 7, Fig. 44).

The embryo shown in Plate 7, Fig. 44 typifies the external appearance of most embryos in the advanced gastrulation stage. In this, the stomodeum has begun its extensive involution which would eventually help to line the cephalopharynx with the head ectoderm. The CF, as an embryonic landmark, was in the process of disappearing as was much of the amnio-serosa. Paired tracheal pits, external surface lines indicating segmentation and cephalic appendages have come into sharper relief.

Internally, about this stage and with the stomodeal involution continuing, the distal part of the gut has started its posteriad retreat; a move that eventually placed the hind gut at its definitive posterior ventral location. Most embryos achieved the last objective by either stage 16 or 17.

On the other hand, Figures 45-48 (Plate 8) represent various views of a post-gastrulation embryo taken at stage 19. It measured 800 by 160u. Its combined proto- and gnatho- cephalon,

with a lateral midline length of 240u, was clearly delineated from the segmented thoraco-abdominal region. This head region had a well defined clypeolabrum ringed off about 70u from the anterior tip, as well as a conspicuous stomodeal opening--near which sections of combined mandibulo-maxillary rudiments may be observed. The salivary gland ducts had arrived and fused at the ventral midline although the labial lobes per se had not yet done so.

Eleven segments (three thoracic and eight abdominal) could be observed. They averaged about 50u in width. The tracheal pits have closed over; a situation that pressaged the eventual formation of tracheal trunks internally through the unification of the internal bases of the original pits (Plate 14, Fig. 71). The posterior spiracle rudiments could be seen on the dorsal aspects of the eighth abdominal segment and terminally the lateral lobes of the anal segment remained separated by a deep median fissure (Plate 8, Fig. 48).

ORGANOGENESIS

SEM studies of the developmental steps in post-gastrulation embryos yielded significant information as regards the appearance, morphology, location, migration and growth of rudiments of definitive structures of the first-stage larva.

Head Involution. The formation of the head of first stage larva involved a composite series of movements of various rudimentary

parts and appendages of the cephalon. Figures 49-57 (Plates 9-11) represent an SEM study of the more significant steps involved.

The major parts and rudiments of the protocephalon and gnathocephalon present at the beginning of these movements have been treated above under gastrulation. Three additional embryonic rudiments were, however, discernible in such stage 19 or 20 embryos: those of the dorsal organ (DO) and terminal organ (TO) occurring on the maxillary segment and the ventral organ (VO) situated on the labial segments (Plate 9, Figs. 49, 51 and 52).

One of the earliest major movements was the medial migration of the labial lobes which at first led to the unification of the rudiments of salivary gland ducts at the ventral midline and also resulted in the fusion of the labial lobes into a median organ. From this median position, an enlarging labium moved cephalad. In its largest undifferentiated form and carrying the ventral organ rudiments, the latter arrived at its final position on the floor of the mouth by stage 22 (Plate 9, Figs. 50 and 51).

Another major development involved the rudiments of the maxillary segments which carried the placodes of the dorsal and terminal sense organs. These laterally situated primodia developed rapidly in post-gastrulation period and elongated cephalad in equal proportion to the labium up to the time of the stage 22 embryo (Plate 9, Figs. 50-52 and Plate 10, Fig. 53). Subsequently the lobes elongated farther across and over the clypeolabrum, to eventually fuse over the midline at the anterior

tip of the embryo at about stage 26 as cephalic lobes (Plate 10, Figs. 54-56; Plate 11, Fig. 57).

Concurrently, a dorsal ridge from the first thoracic segment which usually form the "roof" over the mature larva head when withdrawn, started to develop as a collar over the dorsal posterior aspect of the cephalon at about stage 22 (Plate 9, Fig. 51). The rudiments of this collar scales which eventually cover the anterior ventral and lateral aspects of the first stage larva's second segment (first thoracic), were also evident at the same stage. Two transversely running ventral cephalic bands separated the maxillary from the wide posterior portion of the labial lobe of the head (Plate 10, Figs. 55 and 56). Notably, the ventral organ (VO) had almost completed development on the lateral aspects of the lobe by stage 38 (Plate 10, Fig. 56), whilst at about the same time the mouth hook was protrusible and the DO and TO were assuming detailed structural forms on the now fused cephalic lobes (Plate 11, Fig. 57).

Developmental Processes in the Terminal Abdominal Segments. Significant structural changes were evident on the external surface of the last two (eighth and ninth) abdominal segments during organogenesis as reflections of internal changes. Figures 58-63 (Plates 11 and 12) represent an SEM study of these changes.

As has been noted previously, there was a deep fissure in the stage 19 embryo between the two lateral lobes of the anal segment. Also, in the same embryo, the depression for the future posterior spiracles were located dorso-laterally on the eighth abdominal segment (Plate 8, Fig. 48).

By stage 22, these spiracular depressions have moved medially and dorsally, as shown in Fig. 61 (Plate 12) which also illustrated the eruption of the spiracular rudiment proper. After rounding out terminally as small knobs by stage 26 (Plate 12, Fig. 61) these rudiments regularly budded out four leaflike outgrowths laterally. Each of these outgrowths assumed their definitive four or five-fingered forms by about the twentieth hour of development. In addition, each spiracular tubercle exhibited one slit-like and one round opening on its medial surface when full-grown (Plate 12, Fig. 63).

The taxonomically important anal organ extending laterally around the anus in the first instar larva usually carried a battery of characteristic structures: tubercles, lobes and others (Plate 12, Fig. 63). In addition, its edges were provided with multiple-rowed complement of spines (characteristically also found in all intersegmental abdominal areas) though fewer rows of spines marked the posterior edge than the anterior. The placodes of this organ first made their appearance about the tenth hour of development as two curved ellipsoidal depressions, one on either side of the anal fissure (Plate 11, Fig. 58). By the time of stage 23 embryos, however, the placodes had come into sharper relief with the anal fissure almost completely fused behind them (Plate 11, Figs. 59 and 60). At the level of stage 26 embryo, the perianal lobes of these plates as well as their contoured multi-tuberculi appearance have become largely established. The spinous post-anal tubercle, characteristic of

Stomoxys spp. larvae, was also visible in the thirteenth-hour embryo as well as rudiments of two additional tubercles placed on either side of the former. The spinous elements made their appearances as early as the tenth hour of development (Plate 12, Fig. 62). Post-stage-40 embryos showed all these structures with gross appearances that were largely indistinguishable from those of the hatching larva.

Development of Other Thoraco-Abdominal Structures. Ventral spines were restricted to the abdominal tagma (last eight segments) of the developing larva. They appeared initially, at about the time of stage 22 embryo, in rows along the anterior and posterior ventral edges of each segment except the anterior edge of the first, and posterior edge of the last, abdominal segments (Plate 12, Fig. 64; Plate 13, Fig. 65).

A pair of triclad sensilla developed regularly on the ventro-lateral aspects, and midway through the width, of each thoracic segment (larval segments 2-4) of the hatching larva (Plate 16, Figs. 79 and 80). Their placodes were easily discernible as early as in stage 22 specimen, a time during which rudiments of the anterior spiracles were also seen (Plate 10, Fig. 53). The latter occurred in slit-like depressions on the latero-dorsal aspects of the first 'thoracic' segment of such stages.

The Hatching Larva. Under the conditions of this investigation, the first instar larva hatches at an average of 23 1/2 hours post-oviposition by pushing its head against, and splitting off, the dorsal plate along the hatching lines (Plate 1, Fig. 2).

SEM and light microscopy studies of the main structural features characteristic of the subsequent first stage larva are presented in Figures 66-84 (Plates 13-17).

At hatch each larva is creamy white; the oral hook constituting the only black streak at the anterior end. Each is cone-shaped, with the anterior end narrow, and measures on the average 900u by 170u (Plate 13, Fig. 66). The head is retractable under a collar produced by the first thoracic segment and the posterior ventral portion of the cephalon (Plate 13, Fig. 67).

The larva head presents a number of conspicuous external features, including three pairs of major sense organs: dorsal, terminal and ventral organs (Plate 13, Fig. 68 and Plate 14, Fig. 69). Other minor sense organs or receptors, however, occur on the head, e.g. a para median spot sensillum (Plate 11, Fig. 57) on the cephalic lobes.

The paired dorsal organs are situated dorsally on the head, one on each cephalic lobe about 100 microns from the anterior tip of the larva head. Each organ possesses a dome-shaped sensillum, about 6u in diameter, which is jacketed proximally in a material not-unlike the larval head integument itself (Plate 15, Fig. 74). A lateral dome receptor, partly hidden by the collar, nestles on the lateral aspect of the base of the domed structure.

The paired terminal organs lie more or less at the anterior tip of the head. Enclosed in two whorls of larva head intergument, it measures approximately 11u in diameter (Plate 15,

Fig. 75). It may be divided for description into two portions: a dorso-lateral one-third containing two sensilla--one papilla, the other a modified papilla sensillum; and a ventro-medial portion containing usually ten sensilla--three papilla, two knob, four pit and a spot sensilla.

The paired ventral organs, 2u long, are located on the lateral aspects of the small ventral lobe of the head (Plate 15, Fig. 76). They are each composed of at least four units--one papilla, one pit and two knob sensilla (Plate 16, Fig. 77).

Other important features of the head include a protruscible hook (Plate 11, Fig. 57) and a collar of scales around the ventral and lateral (but not dorsal) aspects of the posterior portion of the cephalon (Plate 14, Fig. 69).

The thoracic and abdominal features of the hatching larva are as previously described under sections on their developments.

Figures 70-73 (Plates 14 and 15) were chosen to illustrate various relationships of the internal organs of the hatching larva as seen under light microscope. Among the main features, the tracheal trunks are seen as two main pipes spanning the length of the embryo, dorsally and under the segmental muscles, from the first 'thoracic' segment to the eighth 'abdominal' segment. The intraoral locale of the mouth hook is obvious. The alimentary tract features well differentiated parts, especially the pharynx, proventriculus, gastric caeca, ventriculus and rectum. Paired salivary glands with relatively long ducts

lie lateral to the foregut whilst the brain (coalesced ganglia) and the bipartite ventral nerve cord are well represented.

DISCUSSION

Allowing for possible species specific requirements necessitating variations and our experimental conditions, the theme obtained from this study of embryonic developments in the stable fly was that its main features belong in the mainstream of classical descriptions of Dipteran embryology; especially as summarized by Anderson (1966) and confirmed or elaborated on since by workers like Davis (1967), Horsfall et al., (1973), Cantwell et al., (1976) and Mahowald and Turner (1978) amongst others.

Observations concerning the external parameters (color, form, markings and dimensions) of the fly's egg made during this study broadly agreed with the earlier descriptions made by Newstead (1906) and Hindle (1914). The provision of fine structural details here would help, therefore, in expanding cryptic statements typical of the older works as well as provide usable data for future investigators.

Working towards the latter objective, SEM studies of the egg surface, for instance, enabled the location and description of features with greater accuracy. Thus, the single micropyle in this fly was observed specifically to perforate the thin, semicircular plate of the shell at the ventro-anterior end of the egg whilst its dimensional details were also obtained. These details should be helpful to investigators interested in micropyles and sperm entry into the egg. It must be noted here that patent micropylar openings occurred in all our specimens,

a situation that might not be 'natural' since if these openings had been plugged in nature by materials removable (dissolvable) by our preparatory procedures, a false patency would thus have resulted.

Beament (1946) noted the hexagonal markings on the surface of the eggs of Rhodnius and postulated that these were stamped impressions left by the follicle cells responsible for secreting the egg shell. Similar markings were found in our specimens but they came in an admixture of tri- to hexa-gonal forms and were larger around the eggs' equator than at the poles. More noteworthy was the observation that when at times the outer layer of the shell gets eroded (by abrasive external conditions or aging), for example as seen on the dorsal plate in Fig. 2, the lines of denudation invariably followed these interplate junctions--most probably because of weak association between adjacent plates.

The cross-sectional study of the egg shell, which was probably the first time this has been done in the stable fly, yielded new information in understanding the hatching process in this species. In this respect, we described here, probably for the first time, two anatomical features: (i) the dorsal plate of the egg shell, which lies between the hatching lines is greatly thickened at the anterior end. This thickness was noticed to decrease posteriorly (Fig. 6) and (ii) the joining of adjacent (dorsal and semicircular) shell plates at the hatching lines effected by an interdigitating arrangement of lamellate

extensions of chorionic material from opposing plate edges, with the lamellate extensions running perpendicular to the long axis of the hatching lines (Fig. 7).

The importance of these developments becomes clear when it is remembered that at hatching time the stable fly larva bursts out by pushing its head up and against the anterior end of the dorsal plate thereby causing a split along the longitudinally running hatching lines (Bishop, 1913; Hindle, 1914). The thickened anterior end of the dorsal plate would, therefore, help in sustaining adequate pressure build-up by comparatively increased weight resistance whilst the perpendicular interdigitation at the hatching lines would facilitate simple "sliding" off of opposing plate edges, especially in the absence of overhanging lips as reported, for instance, in Calliphora eggs (Hinton, 1960).

Further, the descriptive morphology of the different layers of the egg-shell obtained in this study agreed with the generalized morphological picture of the muscid fly egg-shell or plastron given by Hinton (1969) indicating probably a family or subfamily based basic pattern in egg shell microscopic structure occasioned by similarity in ovipositional site preference. On the other hand, we observed no gross surface morphological differences between the dorsal and semicircular plates as was, for instance, noted in Calliphora eggs (Hinton, 1960). The dorsal plate in the stable fly's egg tended to be more prone to erosion of its surface than the semicircular plate (Plate 1, Fig. 2).

With regard to the embryo proper, at oviposition the observed thin periplasm, variously sized yolk spheroids and granules and numerous vacuoles were characteristic Dipteran features. However, the constant, dense, periplasm-based coat of microprojections on the external surface elicited the view that these probably evolved to increase the total surface area available for the conjecturably high respiratory demands on the developing embryo. In this respect it may be noted that Turner and Mahowald (1976), using the SEM, also observed similar microprojections in the non-muscid Drosophila.

Meiotic figures were not observed in our specimens most probably because any reduction divisions would have been completed by the time our stage 0 samples had been fixed. A similar handcap was experienced by Cantwell et al. (1976) in studies involving Musca domestica.

The average cleavage rate of one every eight minutes at 30° C obtained for the early cleavages of these embryos was slower than the rate obtained at the lower temperature of 25° C in Musca by Cantwell, et al. (1976) since temperature decreases tend to slow this process. However, the rate still fell within the range of normally fast cleavage rates characteristic of Cyclorrhaphan embryos (Anderson, 1962). Culex molestus, by comparison has a much slower Nematoceran rate of one every twenty minutes (Christopher, 1960).

Cleavage itself was clearly centrolecithal and proceeded in typical Dipteran fashion of rapid synchronous nuclear divisions

and redistribution, culminating in the nuclear invasion of an enlarging periplasm for an eventual formation of a cellular blastoderm (Anderson, 1966).

Three important, though by no means unique, features were recorded at this stage which must be considered characteristic of the species because of regularity of occurrence: (i) the periplasmic invasion occurred at the ninth cleavage stage, (ii) the primary vitellophages were specifically formed by nuclei (120 $\pm$ 20) remaining in the yolk after the mass nuclei migration to the periplasm, Figs. 19 and 29, and (iii) secondary vitellophages were formed from nuclei reinvading the yolk from the posterior pole--before cytokinesis--Fig. 30.

The ninth cleavage level invasion of the periplasm appeared not to be irregular. In Musca domestica, for instance, Cantwell et al., (1976) noted that the nuclei had not arrived at the periphery at the 'seventh and eighth' cleavage stage whilst Turner and Mahowald (1976) noted that this event occurred at the ninth cleavage in Drosophila melanogaster. On the other hand, the observed mode of formation of primary vitellophages has been previously reported in many Dipterans (Horsfall et al., 1973; Davis, 1967) whilst Sonnenblick (1950) and Anderson (1962) had noted the formation of secondary vitellophages from posterior pole cells in Drosophila melanogaster and Dacus tryoni, respectively.

In SEM studies of the egg surface after nuclear invasion of periplasm it was noted through studies of waves of mitotic

divisions of surface bulges and their attendant increases in number that there were four post-invasion synchronous nuclear divisions. This observation thus brought to 13 the number of cleavages that yielded the final complement of blastoderm cells, a number that has been recorded in Nematoceran Culex molestus (Christopher, 1960) and Cyclorrhaphan, D. melanogaster (Turner and Mahowald, 1976; Zalokar and Erk, 1976).

Further, whilst the distinct cap of cells formed in this species by the posterior pole cells was characteristic of the Diptera (Anderson, 1962), what again may be a species characteristic of an initial complement of nine cells at the time of nuclear invasion of polar plasm was here recorded. These cells subsequently underwent two asynchronous divisions to yield a final complement of 36 posterior pole cells. However, a few extra nuclei have been noticed at various stages whilst at other times full complement may be depleted by reinvasion of the yolk during secondary vitellophage formation. It is of note that Cantwell et al. (1976) recorded an early complement of "above eight nuclei" in Musca domestica.

The time range of 2 to 2 1/2 hours (average 2 1/3 hours) at 30° C taken to complete blastoderm formation in this species compares slightly slower than the 2 1/2 hours at 25° C obtained in Cocchliomyia hominovorax (Reimann, 1965) and Musca domestica (Cantwell et al., 1976) but certainly faster than the five to six hours obtained in Dacus tryoni (Anderson, 1962) indicating probably wide transorder species variations.

In later stages and as expected of a Dipteran, the internalization of the presumptive mesoderm in this species was by invagination rather than 'sinking' of presumptive mesodermal cells from the ventral midline surface (Anderson, 1973). In addition, the description given here of the sequence and mode of formation of the ventral furrow, anterior and posterior midgut rudiments as well as the subsequent stomodeal and proctodeal rudiments along the ventral line were also classically Dipteran (Anderson, 1966).

Some features of this period of embryonic development were, however, stamped with species based traits. Thus, we noted that there were constantly five temporary transverse folds (posterior to the cephalic furrow) ahead of the elongating germ band on the dorsal surface of the embryo (Plate 5, Figs. 34 and 35). They were relatively fixed in their position and time span and tended to disappear in a definite pattern, from the posterior ones anteriorly. In addition, the cephalic furrow was always a deep slanting invagination describing off the anterior third of the embryo.

It is considered here that these features may be assumed species characteristic on account of their regularity, though not species specific since existing literature on these folds emphasized the great variations in these features exhibited among many species (Anderson, 1962). Moreover, after careful study of subsequent segmentation and determining that the mandibular, maxillary and labial rudiments developed posterior to the

cephalic furrow, it may be reasonable to agree with previous workers like Guichard (1971) and Mahowald and Turner (1978) who felt that the cephalic furrow might be involved in the metameric determination of the anterior part of the embryo, particularly the post-oral segments. There was no conclusive evidence suggesting that the remaining folds had anything to do with metameric determinations.

Two other features deserve mention if only to emphasize the Cyclorrhaphan nature of the embryos (Anderson, 1966). Thus the amnioserosa layer was neither extensive nor firmly attached to the underlying yolk sac. Its disappearance (which was finally completed at about the seventh to eighth hours of development) was always obvious ahead of the elongating germ band. Also, in typical Cyclorrhapan fashion the elongating germ band carried at its tip the posterior pole cells in a pocket formed by the migrating posterior mid-gut rudiment. That the mesodermal rod failed to migrate around the posterior tip with the ectoderm during the elongation of the germ band appeared to confirm the view of Turner and Mahowald that the mesoderm may not regularly accompany the ectoderm around the posterior tip of embryo (1977).

Morphological developments as recorded by the SEM in middle and late embryogenesis, helped to locate the origin, time of emergence and stages of development of many organs of the first stage larva probably for the first time. It was noted, for instance, that the salivary glands as well as the ventral (sense) organ of the larva had their paired placodes on the labial

rudiments while the latter were still laterally positioned in the stage 21 embryo. In this respect, it may be noted that Mahowald and Turner (1978) mentioned the salivary gland as originating from the labial segment in D. melanogaster, thus confirming with SEM the previous observation of Ueda and Okada (1977). Neither pair of workers, however, noticed the presence of the ventral organ placode on the same rudiment as noted here.

On the same note, this study confirmed for this species that the dorsal and terminal organs as well as the spot sensillum of the first stage larva originated from the maxillary rudiments. On the other hand, the ventral organ originated from the labium rudiments in this species. Notably Schoeler (1964) working with Calliphora erythrocephala and Bull (1952) working with D. melanogaster had suggested the maxillary origin of larval sense organs. In addition, one must note the preponderant role played by the maxillary lobe in the formation of the two cephalic lobes of the larval head since they grew to cover the lateral, anterior and most of the dorsal aspects of these lobes.

Further, our findings on the detailed structure of the dorsal and terminal organs bore striking resemblance to the ultrastructural descriptions of similar organs in the housefly, Chu and Axtel (1971 and 1972), which may suggest some structural similarity at the family or subfamily level. According to the same authors they have in addition observed complement of the same organs (as well as the ventral organ) in the cephalic lobes of larvae of Sarcophagidae, Calliphoridae and Chloropidae,

another fact suggesting these organs may be typical of higher Diptera. In any case, the more complex structure of the ventral organ of the stable fly larva, with its package of at least four types of sensilla, compared with the single pore form of it in the housefly larva, Bolwig (1946) may reflect either an evolutionary advance of this structure or an adaptation to a specific (undetermined) factor peculiar to the stable fly.

The double tracheal trunks of the stable fly larva are singularly prominent structures. Their terminally located anterior and posterior pair of spiracles are also prominent and constant. The posterior pair singularly possesses characteristic leaf-like appendages not previously described that may prove taxonomically important. The claim that these spiracles are non-functional or rudimentary in first stage Dipteran larva was doubted by Cantwell et al. (1976) and is also here challenged.

The taxonomically important anal organ of the larval stable fly proved in this study to have originated from the ventral ectoderm of the eight abdominal segment as two placodes on either side of the anal fissure that was still patent in the stage 20 embryo. In subsequent stages it acquired a pair of taxonomically important post-anal tubercules (stage 26), closed off the anal fissure, got invested with typical interabdominal-segment spines and developed a few sense organs.

Finally, although no attempt would be made here to draw a fate map for the presumptive areas of the stable fly larva, one

could not help but note that the locations of various primodia have been largely resolved in this study. These include the ventral furrow (presumptive mesoderm/ectoderm); anterior and posterior mid-gut rudiments; the stomodeal and proctodeal rudiments; the cephalic furrow; the post-cephalic-furrow mandibular, maxillary and labial segments; clypeolabrum; dorsal, terminal and ventral sense organs; trachea and the anterior and posterior pair of spiracles; ventral spines; triclad sensilla of the thorax; gonads; salivary glands; mouth hook; extra-embryonic ectoderm, embryonic ectoderm, neuroblasts and the anal organ.

SUMMARY

The egg shell of the stable fly, Stomoxys calcitrans (L), is made up grossly of two plates--dorsal and semicircular--of chorionic material. In cross-section it possesses a wide chorionic layer lying between two air-space mesh-works, the whole structure being covered on the outer and inner side by thin networks of chorionic materials. Aeropyles connect the two air-space mesh-works through the wide middle layer. The anteriorly thickened dorsal plate along with an interdigitating arrangement of chorionic material at the hatching lines facilitates hatching. A single micropyle is present on the ventral anterior end of the semicircular plate.

Cleavage is centrolecithal and proceeds by rapid synchronous nuclear divisions. Nine cleavages are completed before nuclei invade the periplasm followed by four more divisions before cytokinetic processes are instituted to establish the cellular blastoderm at an average of 2 to 2 1/2 hours at 30° C. A syncytial yolk sac is also formed.

Gastrulation involves the formation of a ventral furrow (presumptive mesoderm in process of internalizing under the presumptive ectoderm) and a cephalic furrow. Slightly later comes the formation and internalization of the posterior and anterior mid-gut rudiments which precedes the invagination of the stomodeal and proctodeal rudiments. The anterior and posterior mid-gut rudiments when internalized eventually attach each to the stomodeal and proctodeal rudiments, respectively.

The germ band elongates dorsally and cephalad, ultimately up to the cephalic furrow, pushing ahead of it the posterior mid-gut rudiment and the proctodeal invagination. During its cephalad advance five transverse folds form and disappear and the amnioserosa stretches and thins out ahead and laterad of it. The mesodermal rod spreads to underlie the ectoderm from an internal ventral position and (36 definitive) pole cells migrate inwards in a pocket of the advancing posterior mid-gut rudiment (to eventually form gonads) during organogeny.

Organogeny involves several morphogenetic developments and histodifferentiation. Segmentation, dorsal closure, shortening of the germ band and loss of the amnioserosa are early events. Malphigian tubules differentiate from the proctodeum and the mid-gut join to complete the stomodeum to proctodeum gut tube that later differentiate parts. Head formation is effected by combination of involution of the head ectoderm and movements and concentration anteriorly of the gnathocephalic segments. Differentiation of neuroblasts is from embryonic ectoderm. The larva head sense organs develop from gnathocephalic ectoderm, dorsal and terminal organs from maxillary, ventral organ from the labium. Tracheal trunks form from the internal ends of segmentally arranged tracheal pits and anterior and posterior spiracles form on the first thoracic and last abdominal segments. The anal organ forms from the last abdominal segment.

LEGEND

a = anterior
abl = acellular (syncytial) blastoderm
af = anal fissure
amr = anterior midgut rudiment
an = anus
ao = anal organ
aor = anal organ rudiment
as = amnioserosa
at = anal tubercle
ats = anterior spiracle
bg = (surface) bulges
c = cephalic furrow
cf = cell furrows (or partition)
ch = chorion
chl = cephalic lobe
chp = chorionic plate
cl = clypeolabrum
cs = collar spines
cy = cytoplasm
cyh = cytoplasmic halo
d = dorsal
dp = dorsal plate
dr = dorsal ridge
e = ectoderm
em = embryo

g = gonads

h = hypopharyngeal rudiment

hg = hind gut

hk = (mouth) hook

hl = hatching lines

ia = inner air space of chorion

in = inner (chorionic) network

l = labium

lm = lamellate extension or outgrowths

m = mesoderm

mc = middle chorionic (layer)

me = mesoectoderm

mn = mandibulary (segment)

mpy = micropyle

mt = Malphigian tubule

mv = microvilli

mx = maxillary (segment)

n = nucleus

oa = outer air space of chorion

on = outer (chorionic) network

p = posterior

pat = paraanal (sensilla) tubercle

pl = perilecithal cytoplasmic sac

pm = periplasm

pmr = posterior midgut rudiment

ppc = posterior polar (cap of) cells

ps = posterior spiracle

pv = proventriculus

pvt = primary vitellophages

s = stomodeum

sa = (posterior) spiracle appendage

sd = salivary duct

sg = segmentation

sn = spines

so = spiracular openings

ss = spot sensillium

st = struts or bosses

sv = secondary vitellophages

t = trachea (pits)

tf = transverse furrows

a = anterior furrow

m = median furrow

p = posterior furrow

th = Triclad sensillium

v = ventral

va = vacuoles

vf = ventral furrow

vm = vitelline membrane

vnc = ventral nerve cord

vt = ventricle

x = pharynx

yc = yolk sac

ys = yolk sphere

zn = zygote nucleus

Fig. 1. SEM of the external surface of newly oviposited egg. Note the reticulate markings. X100

Fig. 2. Egg, at point of hatch, showing the (eroded) grooved dorsal surface, micropyle and a split surface of one hatching line. X100

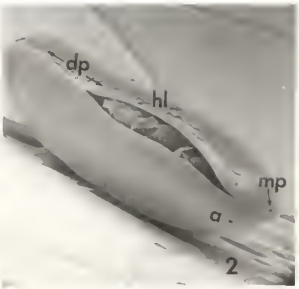
Fig. 3. Anterior end of egg with blown-up micropyle and arched limit of the hatching line. X1,000

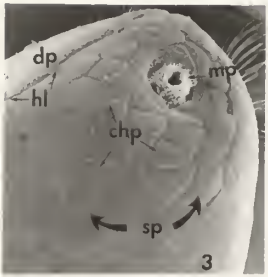
Fig. 4. A cross-section of egg shell. Note the double air space arrangements. X8,000

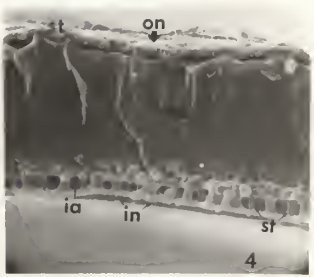
Fig. 5. A view of the dorsal plate outer grid and underlying air space. Note struts exposed by erosion of covering grid. X10,000

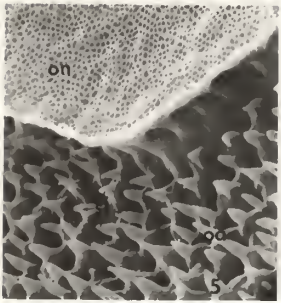
Fig. 6. A transverse cut through the whole of egg shell near the anterior end. Note the relationship of the hatching line, the thickened dorsal plate and the faint halo of micropyle. X300

PLATE 1









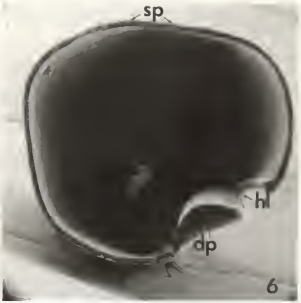


Fig. 7. Higher magnification of hatching line. Note the lamellate chorion and their digitation. X4,000

Fig. 8. Split-egg view showing relationship of chorion, vitelline membrane and embryo substance (at oviposition). X1,500

Fig. 9. A cross-section of split egg at oviposition under transmission-electron microscope. Note the inner air space of chorion (at the upper right-hand corner), the vacuoles and the different sized yolk spheres. (Black marks on the spheres may be artefacts.) The periplasm is thin but discernible. X2,100

Fig. 10. Blunt anterior end of embryo as seen under transmitted light microscopy. Note the reflecting vitelline membrane. X540

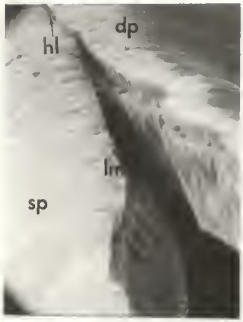
Fig. 11. SEM of the outer surface of the egg plasm at oviposition. Note the dense microprojections, some of which are filiform, others knobbed. X400

Fig. 12. TEM of embryo at stage 0. Note the periplasmic origin of the microvilli, the variously-sized vacuoles and yolk spheres. Note the thin periplasm. X3,000

Fig. 13. SEM of the internal structure of the fractured embryo (stage 0). A three dimensional impression of the spheres and vacuoles may thus be obtained. X2,000

Fig. 14. Stage 2 embryo in cross section. Note the well developed periplasm. The yolk spheres have begun their concentration at the center of the embryo. X400

PLATE 2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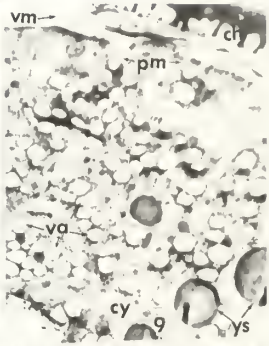
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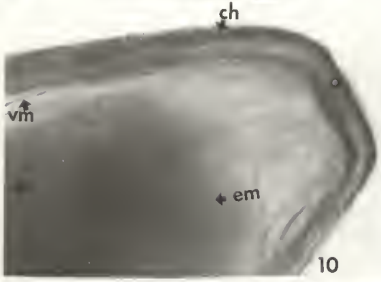
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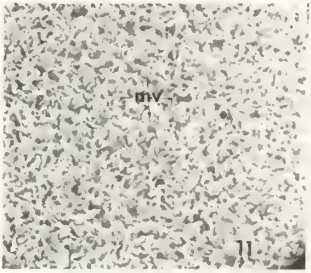
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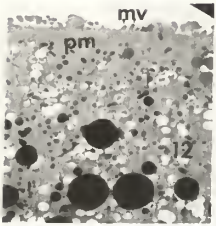
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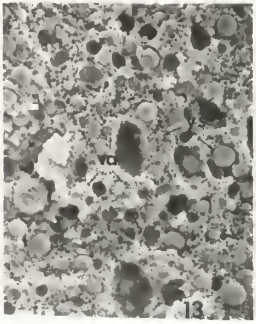
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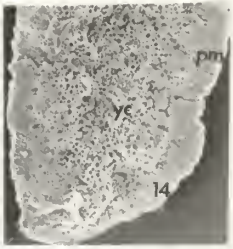
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Fig. 15. TEM of stage 2 embryo showing cleavage nuclei. Note the enveloping cytoplasmic islands around the nuclei. X1,600

Fig. 16. A cross section of stage 2 embryo showing cleavage nuclei arranged around the "cross sectional" center of the embryo. X450

Fig. 17. Stage 2 embryo showing distribution of the cleavage nuclei, along the longitudinal axis of the embryo. X350

Fig. 18. Embryo at the syncytial blastoderm stage showing yolk nuclei and apical surface bulges. X150

Fig. 19. TEM of syncytial blastoderm showing the distribution of nuclei in the periplasm. X3,000

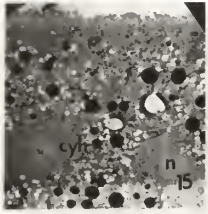
Fig. 20. Post-tenth cleavage embryo showing the characteristic surface bulges. X100

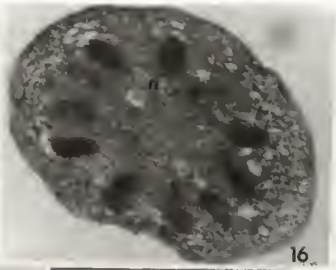
Fig. 21. Embryo at about the twelfth cleavage stage. Note the increase in surface bulges and the distinct posterior polar cap of cells. X100

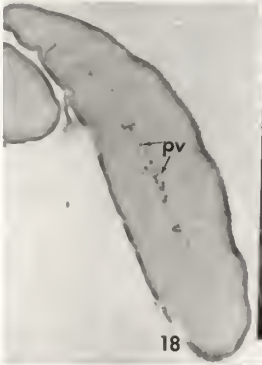
Fig. 22. Thirteenth cleavage embryo with the closely packed surface bulges. Cytokinesis about to start. X100

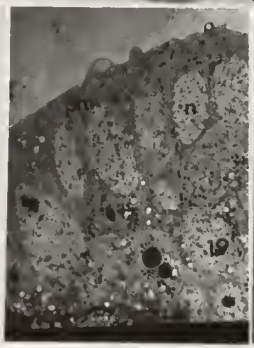
Fig. 23. Embryo in the early stage of cytokinesis. Note the cellular furrow growing in from the apical surface and the concentration of yolk in the center of embryo. X400

PLATE 3









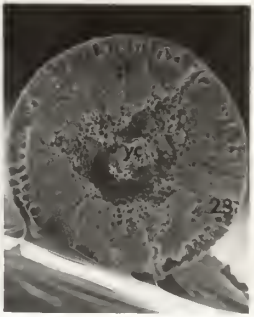


Fig. 24. Posterior pole of embryo from Fig. 21 showing the initial complement of pole cells. Some cells are in the process of dividing. X400

Fig. 25. Posterior pole of thirteenth cleavage embryo showing the final cap of 36 polar cells. X400

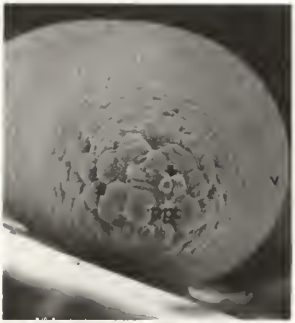
Fig. 26. A section of embryo apical surface showing typical blastoderm and posterior pole cell under high magnification. Note that the posterior pole cells appear larger and possess less microprojections. X2,000

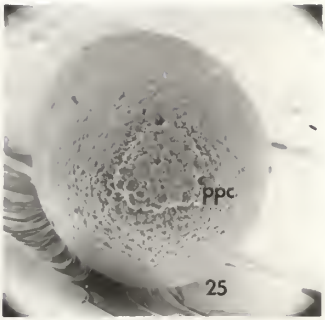
Fig. 27. Longitudinal section of embryo at the syncytial blastoderm stage. Note the yolk nuclei lying at the midline. X540

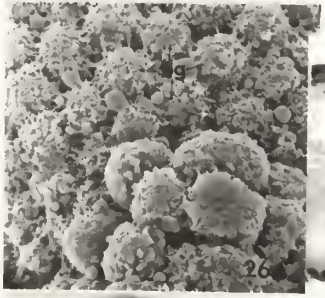
Fig. 28. Secondary vitellophages in formation. Pole nuclei invade the yolk at the midline. X540

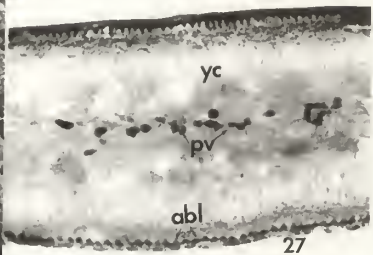
Fig. 29. An SEM cross section of the cellular blastoderm. Note the conspicuous yolk and the perilecithal sac (membrane). X400

PLATE 4









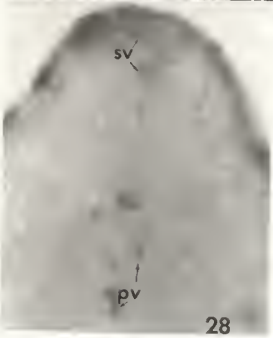


Fig. 30. Fractured embryo showing the start of ventral furrow formation. X400

Fig. 31. Fractured embryo showing the ventral ectoderm, the internal mesoderm and a portion of the yolk sac. X700

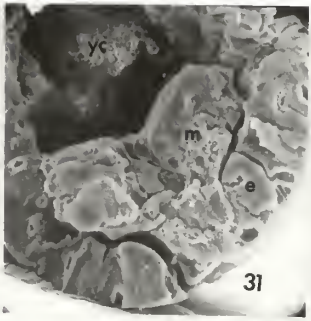
Fig. 32. Longitudinal section of portion of an embryo posterior to the cephalic furrow at the time of the elongation of the germ band. Note the transistory transverse furrows, the pocket formed by the posterior midgut rudiment and the ventral location of mesodermal tissue. X250

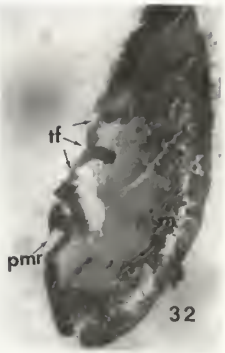
Fig. 33. Longitudinal section through the embryo at the time of the retreat of the hind gut rudiment. Note the stomodeal pocket. X160

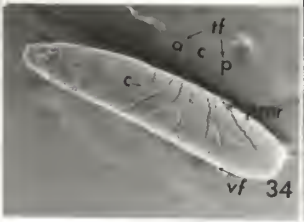
Fig. 34. Embryo showing the full complement of five transistory transverse folds posterior to the cephalic furrow. Note that none of the lines touch the ventral furrow. X100

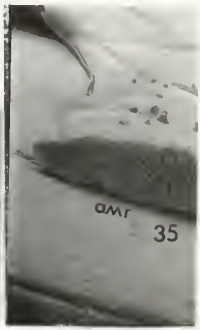
Fig. 35. Embryo of about the same age as the one in Fig. 34; ventrolateral view showing the anterior midgut pocket. X100

PLATE 5









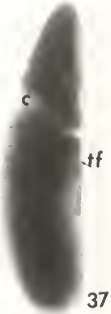
Figs. 36 and 37. Embryos viewed under transmitted light depicting various stages in the formation and disappearance of the transverse lines. Note the deep fissure formed around the anterior end of embryo by the cephalic furrow in Fig. 37. X150

Figs. 38 and 39. Embryos showing the amnioserosa layer in retreat towards the cephalic furrow; ahead of the elongating germ band. Note the midline mesoectoderm and the lateral arms of the amnioserosa. X100

Fig. 40. Ventral view of embryo in late stages of germ band elongation. Note the faint lines of segmentation. X100

PLATE 6

36



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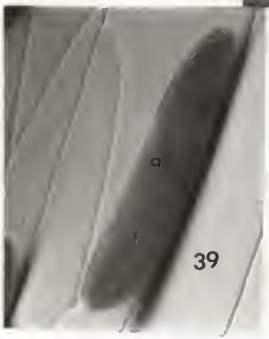

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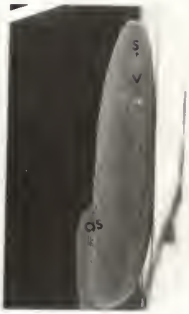


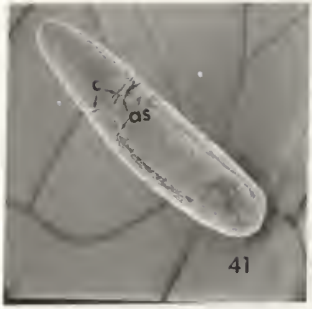
Fig. 41. Dorsal view of embryo. At the time of maximum elongation of the germ band. Note the persistent cephalic furrow and the thin lateral impression of amnioserosa. X100

Fig. 42. Side view of an embryo similar in age to that in Fig. 41 under transmitted light. Note that although there were external lines of segmentation. These were not reflected by the internal tissue. X120

Fig. 43. Side view of an embryo under transmitted light slightly older than the one in Fig. 44. Note the segmentally arranged mesoderm although the germ band has only started its posterior retreat. Note the stomodeal pocket. X100

Fig. 44. Ventrolateral SEM view of a late gastrulation embryo in which external signs of segmentation are much in evidence. Note the laterally placed gnathocephalic segments: mandibular, maxillary and labial, the paired tracheal pits and the stretched amnioserosa.

PLATE 7



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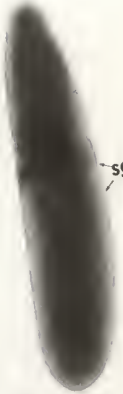

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Figs. 45-48. Different SEM views of an early post-gastrulation embryo.

Fig. 45. Lateral view. Note the clypeo-labrum, the segmented thoraco-abdominal region, the posterior spiracular rudiment. X100

Fig. 46. Ventral view. X100

Fig. 47. Latero-ventral view of the cephalic portion of larva. Note the placodes of the ventral, dorsal and terminal organs; the stomodeal invagination; the prominent mandibulo-maxillary rudiment and the fused salivary gland duct. X300

Fig. 48. Ventrolateral aspect of the posterior region. Note the anal fissure and the posterior spiracular rudiment.

PLATE 8

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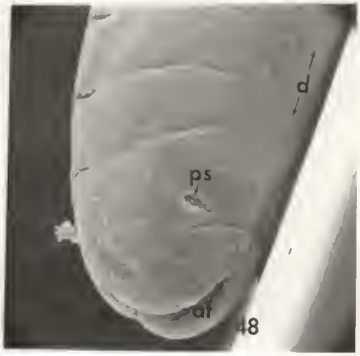

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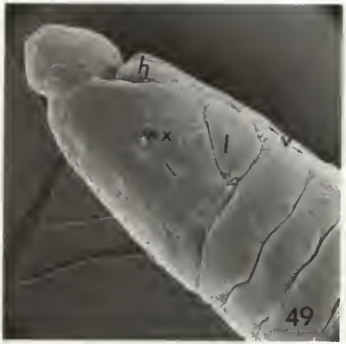
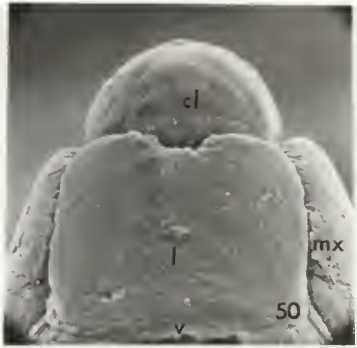
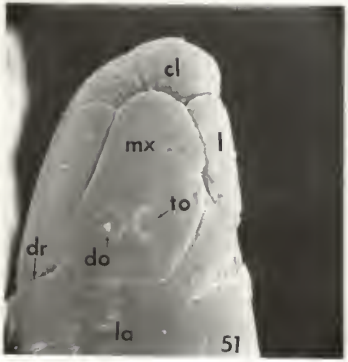
Fig. 49. Cephalic region of embryo similar to Fig. 47 from two different views. X300

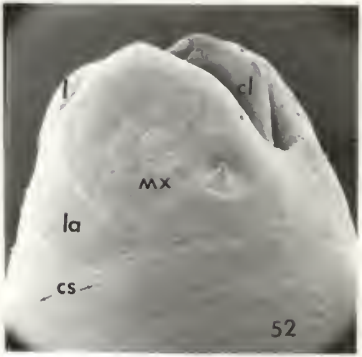
Figs. 50-52. (Fig. 50 x 900) (Fig. 51 x 500) (Fig. 52 x 900) The formation of the head in serial presentation. Note the gradual anterior growth of the labial floor (Figs. 50 and 51) and the contemporaneous growth and subsequent elongation of the lateral head lobe (largely the maxillary segment) to cover the clypeolabrum laterally and dorsally.

PLATE 9



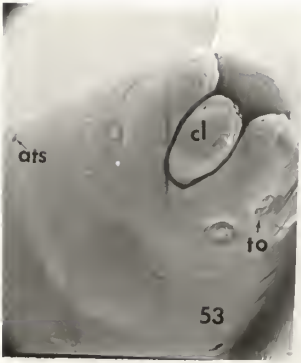


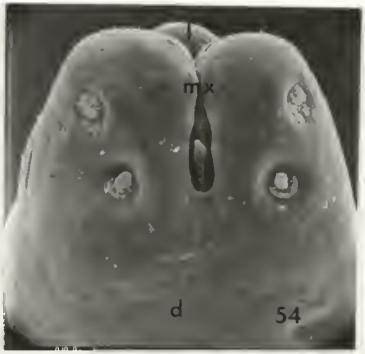


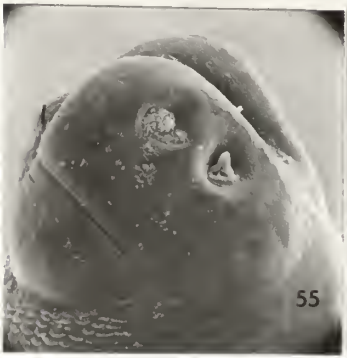


Figs. 53-56. (Fig. 53 x 700) (Fig. 54 x 900) (Fig. 55 x 900) (Fig. 56 x 900) The formation of the head in serial presentation continued. Note the gradual differentiation of the dorsal and terminal organs, the spot sensillium on the cephalic lobes and the ventral organ on the labial lobe.

PLATE 10







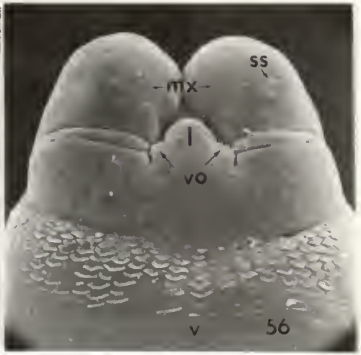
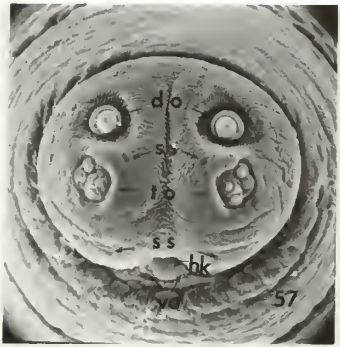


Fig. 57. Anterior view of the nearly hatched larva.

Fig. 58-60. The formation of the anal organ and posterior spiracle in series. Note the early and fissure, anal organ placodes and posterior spiracle rudiments (Fig. 58). The subsequent appearance of rudiments of the ventral spines on closure of anal fissure are depicted in Figs. 59 and 60. (Fig. 58 x 700; Fig. 59 x 700; Fig. 60 x 400).

PLATE 11



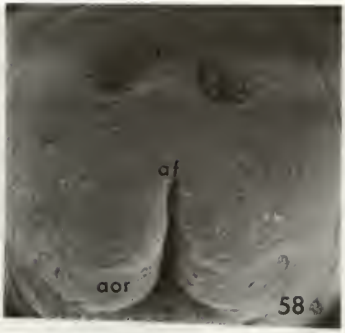


Fig. 61-63. Formation of the anal organ and posterior spiracle continued. Note the late differentiation of the spinous and tubercle (Fig. 61) followed later by the paraanal tubercles (Fig. 62). Note also the relatively later differentiation of spiracular leaflets (Fig. 63). (Fig. 61 x 400; Fig. 62 x 600; Fig. 63 x 400).

Fig. 64. Ventral surface of embryo at time of anal fissure closure, depicting the first appearance and location of the ventral (abdominal) spine rudiments. X200

PLATE 12

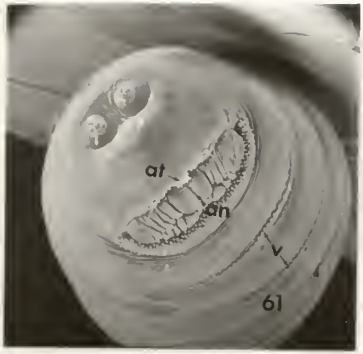


Fig. 65. Ventral surface of an almost mature embryo with the fully developed ventral spines. X160

Figs. 66-68. The external morphology of the first stage larva. The dorsal view of the hatched larval with the head extended (Fig. 66). The anterior end of the embryo with the head retracted (Fig. 67) and extended (Fig. 68). (Fig. 66 x 100; Fig. 67 x 1,000; Fig. 68 x 600).

PLATE 13

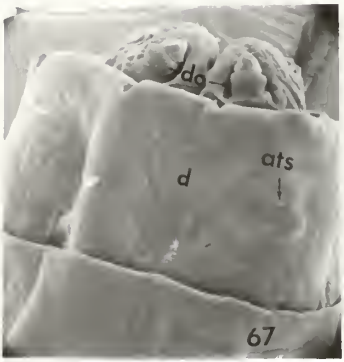


Fig. 69. Ventral view of the extended head. Note the trichoid sensilla on the first 'thoracic' segment. X600

Fig. 70. Frontal section of larva showing internal organs. X100

Fig. 71. Longitudinal section of larva x 120. Note the tracheal trunk, concentrated nervous system and mouth hook.

Fig. 72. Cross section at the anterior end of larva. Note the circumoesophageal disposition of nervous tissue and the paired salivary ducts. X540

PLATE 14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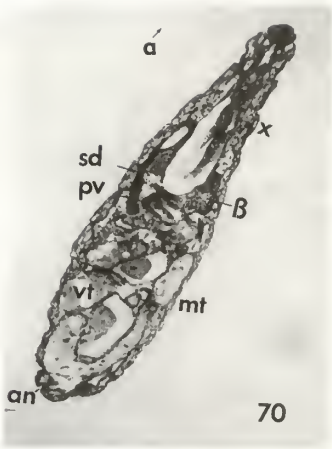
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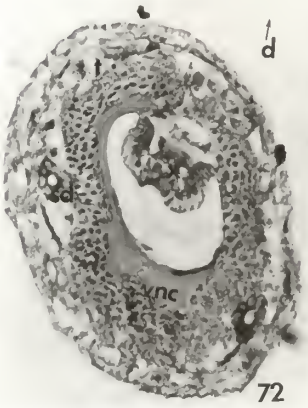
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Fig. 73. Cross section towards the posterior end. Note the gonad, malphigian tubule, tracheal trunks, hind gut and the paired ventral nerve cord. X540

Fig. 74. SEM of the dorsal organ. X4,000

Fig. 75. SEM of the terminal organ. X4,000

Fig. 76. Labial lobe showing the ventral organ. X3,000

PLATE 15

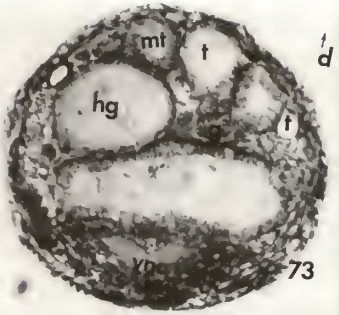


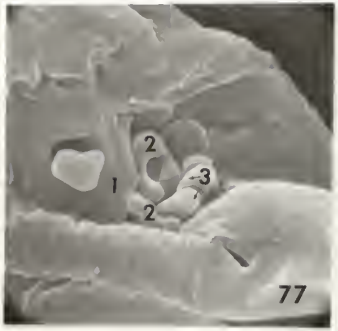
Fig. 77. The ventral organ enlarged showing the various sensilla. X15,000

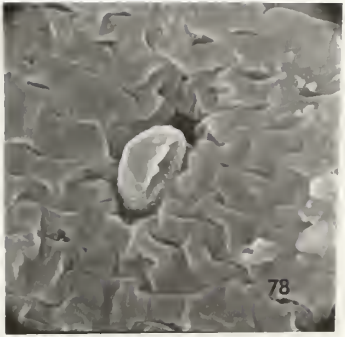
Fig. 78. Anterior spiracle on the first segment. X15,000 Note the plug and the opening.

Fig. 79. Trichoid sensilla of thoracic segments. X8,000

Fig. 80. Embryo with head retracted. Note the paired trichoid sensilla, spot sensilla and mouth depression. X1,000

PLATE 16





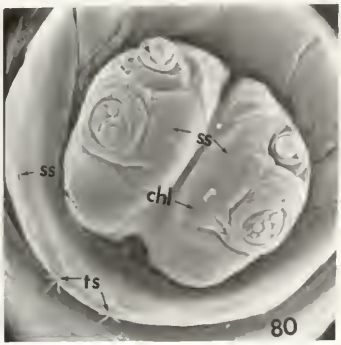


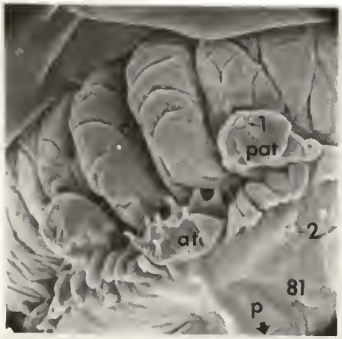
Fig. 81. Anal organ enlarged around the anus. Note the post-anal spinous tubercle, the paraanal tubercules with their terminal knob sensilla and other sensilla in the region immediately behind the anal plate margin. X1,500

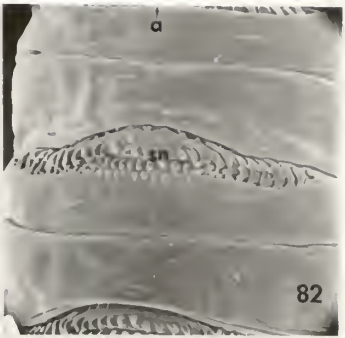
Fig. 82. Ventral surface of larva showing the spinous complement. X600

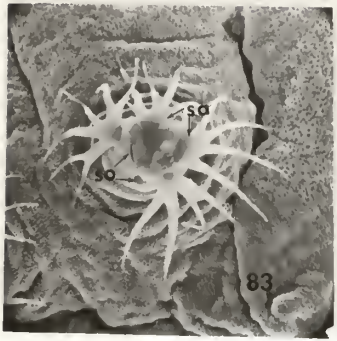
Fig. 83. The posterior spiracle. Note the four leaf-like appendages, the slit and round openings on the tubercle. X1,500

Fig. 84. The ventral posterior surface with the anal organ in view. X400

PLATE 17







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APPENDIX I

Regressive Hematoxylin and Eosin Stain Procedure

Toluene: Two min.

Toluene/100% alcohol: Two min.

Absolute alcohol: One min.

Absolute alcohol: One min.

95% alcohol: One min.

70% alcohol: One min.

Distilled water: One min.

Hematoxylin: Three min.

Distilled water: Rinse - dip several times

Acid water (a few drops of HCL) (or ethanol): Dip to differentiate into red wine color

Distilled water: Rinse

Ammonia water (or lithium carbonate): Dip to blue tissues. Intensity of color should be examined under microscope.

Distilled water: Two min.

80% alcohol: One min.

Eosin Y: 20 sec.

95% alcohol: One min.

95% alcohol: One min.

100% alcohol: One min.

100% alcohol: One min.

Toluene/100% alcohol: Two min.

Toluene: Two min.

APPENDIX I (cont)

Toluene: Two min.

Mount with Histoclad^{R*}

*Histoclad is the trademark of Clay Adams Division, Becton Dickinson and Company, Parsipany, New Jersey.

APPENDIX II

Composition of Some Fixatives and the Buffer Solution

(i) Preparation of 3% glutaraldehyde in 0.1M cacodylate buffer at pH 7.2

$Na(CH_3)_2\ AsO_2 \cdot 3H_2O$ . . . . . 5.35 g

dissolve in . . . . . 200 ml dist. H_2O

adjust to pH 7.2 . . . . . with conc. HCL (drop by drop)

add glutaraldehyde (25%) . . . . . 30 ml.

readjust pH with conc. HCL and dilute to 250 ml

Note: Before adding the glutaraldehyde, it should be shaken in a graduated cylinder or mixed in a beaker with an excess of $BaCO_3$ and filtered clear.

The fixative should be allowed to stand in glass stoppered bottle overnight and then filtered clear again, if necessary. Keep the fixative in a brown glass bottle in a cold room.

(ii) Preparation of 1% osmium tetroxide in 0.1M cacodylate buffer at pH 7.2

(a) $Na(CH_3)_2AsO_2 \cdot 3H_2O$ . . . . . 2.14 g

dissolve in . . . . . 45 dist H_2O

add conc. HCL . . . . . .drop by drop until pH is 7.2

dilute to . . . . . 50.0 ml.

(b) Os_2O_4 . . . . . 1 gm.

dist H_2O . . . . . 50 ml.

APPENDIX II (cont)

Combine the 50 ml of 0.2M buffer solution (a) with the 50 ml of 2% Os_2O_4 solution (b).

(iii) Preparation of 250 ml of the 0.2M sucrose solution, buffered to pH 7.2 with 0.1M cacodylate buffer.

$Na(CH_3)_2AsO_2 \cdot 3H_2O$ . . . . . 5.35 g

dissolved . . . . . 225 ml dist. H_2O

add drop by drop conc HCL . . . . . to pH 7.2

add 17.125 g sucrose

dilute to . . . . . 250 ml.

APPENDIX III

Preparation of Some Stains and Formvar

(1) Preparation of Formvar

(i) Weigh out 0.5 gm of Formvar powder

(ii) Dissolve in 50 ml of chloroform

(iii) Dilute to 0.15-0.25 ml% (with chloroform)

(iv) Store in a brown glass stoppered bottle

(2) Preparation of uranyl acetate

(i) Prepare a 5% solution in 50% ethyl alcohol using glass distilled water

(ii) Adjust to pH 5.0 with dilute NaOH

(iii) Filter through a Whatman #50 paper or equivalent

(iv) Store in a brown bottle away from light

A precipitate will slowly develop, but it will sink to the bottom. Do not disturb sediment during use.

(3) Preparation of Reynold's (1963) lead citrate

Place in a very clean 50 ml volumetric flask with glass stopper:

1.33 g $Pb(NO_3)_2$

1.76 g $Na_3(C_6H_5O_7)\ 2\ H_2O$

30 ml freshly boiled cooled distilled water

Stopper and shake vigorously for one minute and intermittently for 30 minutes. The solution will become milky. Add 8.0 ml of 1N NaOH freshly prepared with boiled distilled water. The solution will become clear. Dilute to 50 with freshly boiled

APPENDIX III (cont)

distilled water. This solution is ready for use. The pH should be about 12.0.

EMBRYONIC DEVELOPMENT OF THE STABLE FLY, *STOMOXYS CALCITRANS*. LINNAEUS (DIPTERA: MUSCIDAE)- A LIGHT AND ELECTRON MICROSCOPY STUDY

by

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D.V.M.; University of Ibadan, Nigeria, 1973

AN ABSTRACT OF A MASTER'S THESIS

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ABSTRACT

Embryonic development of the stable fly, Stomoxys calcitrans (Linnaeus) (Diptera: Muscidae) as studied by light and electron microscopy is hereby described. Cleavage was centrolecithal and by synchronous nuclear divisions whose rate gradually slowed down to yield a cellular blastoderm at about two and a half hours postoviposition and thirteen divisions. Gastrulation was subsequently completed at about eight and a half hours. It involved formation and elongation of a meso-ectodermal germ band, invaginations of stomodeal, anterior and posterior mid-gut and proctodeal rudiments was well as formation of a cephalic furrow and five species-characteristic transistory furrows. Descriptions of surface morphological developments, during organogeny, is given especially for the formation of the larval head, the cephalic sense organs, the anal organ and the spiracles. Average developmental-time to hatch of the embryo was 23 1/2 hours at 30° C and 70% relative humidity.

In addition,descriptive accounts of egg shell structure (as it relates to the hatching and respiration of the embryo) and the surface ultrastructure of the first instar larva are given.